REMARKS

Claim Objections

Claims 19, 21, 26 and 31 were rejected as being in improper form. These claims have been amended to remove all multiple dependencies based on multiple dependent claims.

Claims 2-6, 8-14, 17-32 and 55 were objected to because they contain nonelected matter, subject to traverse. The claims have been amended to remove references to nonelected matter.

Claim Rejections – 35 USC 112

Written Description Rejections

The Applicants acknowledge that the Examiner considers the specification to be enabling for nucleic acids that encode polypeptide SEQ ID NO:2. Claims 1-14 and 17-32 were rejected under 35 USC 112 on the basis that the application does not provide enablement for nucleic acids with homology to those nucleic acids or that encode fragments of a transporter.

The application includes AtNHX1-4, each of which are DNA sequences encoding fully functional vacuolar antiports. The provision of four vacuolar antiports provides strong guidance as to how to make and/or identify other antiports with activity similar to nucleic acid SEQ ID NO:1 and polypeptide SEQ ID NO:2. The percentages of amino acid sequence identity of AtNHX2-4 to AtNHX1 are as follows[1]: AtNHX2: 56.3, AtNHX3: 31.7. AtNHX4: 28.0. The percentages of DNA sequence identity of AtNHX2-4 to AtNHX1 are as follows: AtNHX2: 59.6%, AtNHX3: 34.8%, AtNHX4: 23.1%. This indicates that many variations are possible without impairing antiport activity. Figure 2b provides an amino acid alignment of AtNHX1-AtNHX3. Figure 2c provides an amino acid alignment of AtNHX3 and AtNHX4. These alignments clearly indicate the conserved amino acids in each sequence. This provides guidance as to which nucleotides may be changed to obtain a functional antiport amino acid sequence. If one obtained a DNA sequence that encoded many similarly conserved amino acids, it would be more likely to be a functional antiport . For example, AtNHX1-3 each include a LLPPIIF region (second row of sequences, figure 2b). The amino acids immediately on either side of this region also tend to be conserved. Thus, this provides guidance to retain DNA encoding these amino acids rather than replace them with DNA encoding other amino acids.

[1] Sequence identity was determined in accordance with the method in the patent application: sequences were aligned using the Clustal W program using default parameters (fixed gap penalty=10, floating gap penalty=10, protein weight matrix BLOSUM62).

The application also provides guidance as to the nature of particular domains within SEQ ID NO:1. It includes 12 transmembrane domains, a conserved amiloride-binding domain, and a relatively hydrophilic C-terminal region (page 21, lines 24-28). AtNHX1 shows some similarity at the amino acid level to Na^+/H^+ exchangers isolated from a variety of organisms ranging from yeast (about 27% identity) to humans (about 20%).

Specific guidance on acceptable modifications to nucleic acid and polypeptide sequences is explicitly provided in the application on page 30.

The application includes assays that may be used to readily identify functional vacuolar antiports by experiments with heterologous eukaryotic expression systems and transgenic plants (Example 8). These assays would not involve undue experimentation.

The application also provides a test for a nucleic acid molecule having Na^+/H^+ antiport transporter activity (page 30, beginning on line 24) - determining if the polypeptide produced by the nucleic acid molecule displays the following characteristics: the polypeptide mediates the proton-dependent sodium transport and sodium-dependent proton transport in intact cells, isolated organelles and purified membrane vesicles. These sodium/proton movements should be higher, (preferably at least about 50% higher and most preferably at least about 100% higher) than the proton movements observed in the presence of a background of potassium ions and/or other monovalent cations.

The applicants provide guidance on how to obtain homologous Na^+/H^+ antiport nucleic acid molecules in Examples 2 and 7.

Indefiniteness Objections

Claims 1, 2, 4, 5, 7, 12, 26 and 53 have been amended to remove the term "capable of ..." and replace it with language, such as "provides" that is clear that increased salt tolerance is necessary for the invention, rather than just providing the "capability" of providing salt tolerance.

The terms "include" and "includes" in claims 28 and 29 have been replaced with "comprises".

References to Tables in claims 3 and 23 have been replaced with information from the tables.

To provide an antecedent for "the host cell" in claims 28 and 29, the dependency has been amended to claim 26.

"Nucleic molecule" has been amended to "nucleic acid molecule" in claim 2, part b.

The term "enhances" has been replaced with "provides" in claim 32.

It is submitted that the term "elevated" in claim 32 is not indefinite. The claim is for elevated polypeptide "relative to a non-transgenic plant". The application provides assays for determining the amount of polypeptide and such assays are also well known in the art. Figure 7 also provides a simple and dramatic test for measuring elevated polypeptide levels by measuring salt tolerance in transgenic plants. For the same reasons, it is submitted that the term "increased" in claims 12, 53 and claims 1, 2, 4, 7 and 53 is not indefinite.

The Invention

There is a clear need to provide salt tolerant agricultural plants. Salinity is one of the most serious factors limiting the productivity of agricultural crops throughout the world. In some cases, soils are inherently high in salt. Other soils require vast amounts of irrigation to become productive. Since irrigation water contains dissolved salts and minerals, an application of water is also an application of salt that compounds the salinity problem.

Conventional breeding techniques and genetic engineering for salt tolerance have been attempted by others in order to provide salt tolerant agricultural plants. Both approaches have, to date, been unsuccessful. Salinity remains a major problem. The Applicants are not aware of a single commercially-available plant that overcomes the soil salinity problems outlined above.

The invention in this patent application provides the solution to the soil salinity problem. The invention includes salt tolerant transgenic plants and the nucleic acid molecules and polypeptides that confer this salt tolerance. By way of example, the Applicants direct the Examiner to the dramatic and unexpected salt tolerance of the plants in Figure 7. The scientific community's recognition of the importance of this invention is shown by the publication of the invention in the highly-regarded journal, *Science*[2], in August 1999. In an industry publication (copy enclosed), Dr. Michael C. Shannon, head of the US Salinity Laboratory, stated in regard to the invention that, "This is certainly one of the most interesting accomplishments in the field of

salinity tolerance in the last two or three decades.[3]" The inventor, Eduardo Blumwald, has had subsequent research published in *Nature Biotechnology*[4] (copy enclosed). This article shows the effectiveness of the salt tolerance genes in transgenic tomato plants.

The Examiner's specific objections are addressed in more detail below.

The Applicants submit that the enclosed claims are novel and inventive. There are clear structural and functional differences between the sequences cited by the Examiner and those claimed in the application.

Anticipation - Claim rejections - 35 USC 102

Brant et al.

Claims 1-14, 17, 19-20 and 26 were rejected as anticipated by Brant et al., which discloses a human Na^+/H^+ transporter. This does not anticipate the present claims which are directed to a plant transporter. "PNHX" is defined on page 23, lines 17-19. Brant et al. provides no suggestion of how to obtain plant transporter nucleic acid molecules in order to make transgenic plants.

A person of skill in the art, in possession of this sequence, would not be able to make transformed plants or seeds with altered Na^+/H^+ transporter expression. This reference discloses a truncated fragment of a gene, with no explanation of its function. The fragment does not encode a functional Na^+/H^+ transporter polypeptide. There is nothing in this reference to suggest that the sequence has any relationship to salt tolerance, or to suggest to, or motivate one of ordinary skill to use such a sequence to produce transgenic plants with superior salt tolerance.

Sieyaku et al.

Claims 1-14, 17, 19-20 and 26 were rejected as anticipated by Sieyaku et al, which discloses a rabbit Na^+/H^+ transporter. This does not anticipate the present claims which are

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[2] Apse, M., Aharon, ,G., Snedden, W., Blumwald, E. "Salt tolerance conferred by overexpression of a vacuolar Na+/H+ antiport in *Arabidopsis*." (1999) *Science* 285:1256-1258.
[3] "Botanists design plants with a taste for salt," Chemical & Engineering News (August 23, 1999).
[4] Zhang, HX and Blumwald, E. "Transgenic salt tolerant tomato plants accumulate salt in foliage but not in fruit." (2001) *Nature Biotechnology* 19:765-768.

directed to a plant transporter. It provides no suggestion of how to obtain plant transporter nucleic acid molecules in order to make transgenic plants.

For the same reasons as provided with respect to Brant et al., a person of skill in the art, in possession of this sequence, would not be able to make transformed plants or seeds with altered Na^+/H^+ transporter expression.

Dante M. et al.

The Examiner has alleged that Dante et al. anticipates 1-14, 17, 19-20 and 26. Dante et al. allegedly discloses an *A. thaliana* protein similar to a Na-/H- exchanger in SEQ. ID. NO: 2. Dante et al. does not disclose a nucleic acid molecule sequence.

The Applicants submit that the rejected claims are novel over Dante et al. *There is no polypeptide in Arabidopsis which has the sequence of* Dante et al. Dante et al. discloses a predicted amino acid sequence generated using a computer program. The sequence in Dante et al. is incorrect (perhaps because the prediction program erred in identifying introns and exons). There is no indication that the authors of Dante et al. ever possessed an isolated and cloned sequence. Dante et al. does not suggest how to successfully isolate the sequence. It is not an enabling disclosure of isolated polypeptides and nucleic acid molecules from *Arabidopsis thaliana*.

The Dante et al. sequence includes three regions that are not present in the AtNHX1 polypeptide (amino acids 228-240, 257-264 and 451-457). The Dante et al. sequence also lacks 158 C-terminal amino acids found in AtNHX1. If such a polypeptide was ever synthesized, these changes could potentially alter its stability, regulation and localization.

Dante et al. is described as "similar to sodium/hydrogen exchanger". This is merely speculation, likely based on sequence similarity. No proof of function is provided in Dante et al.. If the authors of Dante et al. had any reasonable basis to conclude that the sequence was a sodium/hydrogen exchanger, the sequence would have been described as an exchanger, rather than as similar to an exchanger. The Applicants submit that a person skilled in the art would recognize that it is very possible that the Dante et al. sequence could have had another function, such as that of a lithium/hydrogen exchanger (lithium and sodium exchangers are often similar).

There is also no suggestion that Dante et al. could produce salt tolerant plants or be a vacuolar exchanger.

A person skilled in the art would not have a reasonable expectation of producing a salt tolerant plant based on the Dante et al. sequence. Thus, Dante et al. would not suggest to, or motivate a skilled person to combine Dante et al. with other art. In view of the error in this sequence and the well known inaccuracies with protein prediction programs, a skilled person would not have a reasonable expectation of success in isolating the nucleic acid molecules and preparing transgenic plants.

Hahnenberger et al. and Young et al.

The research in these references is based on work with a putative yeast plasma membrane Na^+/H^+ transporter (sod2). The Applicants' results are based on work with a plant vacuolar transporter. The exchanger in these references has no sequence identity to the transporter of the invention.

Obviousness - Claim Rejections - 35 USC 103

The Applicants acknowledge the Examiner's finding that claim 55 for SEQ ID NO:1 is free of the art.

The Examiner has objected to the inventiveness of the claims. It was alleged that many Na^+/H^+ antiporters were known in the art and that their use in developing salt tolerant plants was also known in the art.

None of the cited art disclose an isolated plant Na^+/H^+ transporter or a salt tolerant plant including a plant Na^+/H^+ transporter gene. In fact, many persons skilled in the art were of the view, prior to this invention, that salt tolerance could not be conferred on plants by a single gene. In one reference (Rausch et al.; already submitted to the Examiner), the authors state at page 1, column 1 that, "Plant breeding has confirmed that salt tolerance is not conferred by a single trait, but is the consequence of complex gene interactions (Cheeseman, 1988, Bartels and Nelson 1994). As a result progress in understanding the network of molecular mechanisms leading to salt tolerance has been slow (Flowers et al., 1977; Greenway and Munns, 1980; Cheesman, 1988, Munns, 1993)." Clearly, the prior art has many conflicting teachings which lead away from the

present invention. Other articles, such as Schachtman et al. (already submitted to the Examiner), suggest the importance of plant salt tolerance genes, but no article discloses a Na^+/H^+ transporter or a salt-tolerant, transgenic plant. Figure 7 dramatically shows that the present invention answers this long-felt need.

The Applicants submit that none of the cited art discloses isolated nucleic acid molecules suitable for preparation of salt tolerant transgenic plants. As stated earlier, salinity remains a major problem in agriculture. The Applicants are not aware of a single commercially-available transgenic plant that overcomes the soil salinity problems. Some of the art cited by the Examiner presumes the existence of a Na^+/H^+ transporter, but none of the references discloses an isolated Na^+/H^+ plant transporter gene or a transgenic plant including such a gene.

Prior to this invention, there had been some biochemical characterization of putative plant Na^+/H^+ transporter activity. For example, the prior art discloses measurement Na^+/H^+ transporter activity of sugar beet cell suspensions. The art also discloses physiological data about tonoplast Na^+/H^+ transporter activity. However, research using cell extracts merely suggest the existence of a Na^+/H^+ transporter and its activity. It does not provide any suggestion of how to isolate and clone the nucleic acid molecule encoding a Na^+/H^+ transporter or how to prepare transgenic plants. Researchers, despite many efforts, were unable to isolate Na^+/H^+ transporter polypeptides from extracts because there are very few transporters in cell extracts. Thus, the prior art cited by the Examiner only further *emphasizes the need* for the salt tolerance genes and plants of the invention.

Young et al. in view of Gordon-Kamm et al.

The research in Young et al. is based on work with a putative yeast plasma membrane Na^+/H^+ transporter (sod2). The Applicants' results are based on work with a plant vacuolar transporter. As mentioned above, the exchanger in Young et al. has no sequence identity to the transporter of the invention.

One skilled in the art would not be motivated to combine Young et al. with the teachings of Gordon-Kamm et al. to obtain salt tolerant plants. There is no reasonable expectation of success in obtaining a salt tolerant plant by transforming plants with sod2. There have been no

publications by Young et al. disclosing successful creation of a sod2 transgenic salt tolerant plant.

In summary, the Applicants have for the first time isolated and cloned a plant Na^{+}/H^{+} transporter capable of conferring salt tolerance on a cell. They have also determined that the transporter is vacuolar. Its activity has been unambiguously shown in intact vacuoles. None of the prior art has provided the dramatic results obtained by the Applicants, shown in Figure 7 and published in *Science*. Applicants' claims are novel and inventive over the references cited by the Examiner.

The Applicants respectfully request withdrawal of the claim rejections and allowance of the enclosed claims.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "**Version with markings to show changes made.**"

Reconsideration and allowance is respectfully requested.

Respectfully submitted,

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AMENDMENT — VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Claims

Please amend claims 1-4, 6-8, 12, 19, 21, 23, 26, 28, 29, 31, 53, and 55 as follows:

1. (Amended)

An isolated nucleic acid molecule encoding a PNHX transporter polypeptide, or a fragment of a polypeptide having Na$^+$/H$^+$ transporter activity [and capable of increasing] that provides increased salt tolerance in a cell, wherein said nucleic acid molecule is not the sequence having GenBank Accession No. AF007271.

2. (Amended)

An isolated nucleic acid molecule encoding a TNHX transporter polypeptide, PNHX transporter polypeptide, or a fragment of a polypeptide having Na$^+$/H$^+$ transporter activity that provides increased [and capable of increasing] salt tolerance in a cell, comprising a nucleic acid molecule selected from the group consisting of:

(a) a nucleic acid molecule that hybridizes to all or part of a nucleic acid molecule shown in [SEQ ID NO:1], [[SEQ ID NO:3], [SEQ ID NO:17], [SEQ ID NO:19],] or a complement thereof under moderate or high stringency hybridization conditions, wherein the nucleic acid molecule encodes a TNHX transporter polypeptide, a PNHX transporter polypeptide or a polypeptide having Na$^+$/H$^+$ transporter activity and capable of increasing salt tolerance in a cell;

(b) a nucleic acid molecule degenerate with respect to (a), wherein the nucleic acid molecule encodes a TNHX transporter polypeptide, a PNHX transporter polypeptide or a polypeptide having Na$^+$/H$^+$ transporter activity and capable of increasing salt tolerance in a cell.

3. (Amended)

The nucleic acid molecule of claim 2, wherein the hybridization conditions comprise moderate or high stringency conditions, wherein the moderate stringency conditions are 40-50 degrees Celsius, 5xSSC, 2% SDS; wash: 50 degrees Celsius, 0.1xSSC, 0.1% SDS [selected from condition] and wherein the high stringency conditions are 55-65 degrees Celsius, 5xSSC, 2% SDS; wash: 60-65 degrees Celsius, 0.1xSSC, 0.1% SDS [about those in Table 4].

4. (Amended)

An isolated nucleic acid molecule encoding a TNHX transporter polypeptide or a PNHX transporter polypeptide, or a fragment of a polypeptide having Na^+/H^+ transporter activity and that provides increased [capable of increasing] salt tolerance[s] in a cell, comprising a nucleic acid molecule selected from the group consisting of:

(a) the nucleic acid molecule of the coding strand shown in [SEQ ID NO:1], [[SEQ ID NO:3], [SEQ ID NO:17], [SEQ ID NO:19]] or a complement thereof;

(b) a nucleic acid molecule encoding the same amino acid sequence as a nucleotide sequence of (a); and

(c) a nucleic acid molecule having at least [17]30% identity with the nucleotide sequence of (a) and which encodes a TNHX transporter polypeptide or the PNHX transporter polypeptide or a polypeptide having Na+/H+ transporter activity.

6. (Amended)

The nucleic acid molecule of claim 1, comprising all or part of a nucleotide sequence shown in [SEQ ID NO:1], [[SEQ ID NO:3], [SEQ ID NO:17], [SEQ ID NO:19],] or a complement thereof.

7. (Amended)

An AtNHX nucleic acid molecule isolated from *Arabidopsis thaliana*, or a fragment thereof encoding a transporter polypeptide having Na^+/H^+ transporter activity that provides increased [and capable of increasing] salt tolerance in a cell.

8. (Amended)

A recombinant nucleic acid molecule comprising a nucleic acid molecule of any of claims 1 to 4 and a constitutive promoter sequence or an inducible promoter sequence, operatively linked so that the promoter [enhances] provides transcription of the nucleic acid molecule in a host cell.

12. (Amended)

The nucleic acid molecule of any of claims 1 to 4, wherein the TNHX transporter polypeptide or the PNHX transporter polypeptide [is capable of extruding] extrudes monovalent cations out of the cytosol of a cell to provide the cell with increased salt tolerance, wherein the monovalent cations are selected from at least one of the group consisting of sodium, lithium and potassium.

19. (Amended)

A host cell comprising the recombinant nucleic acid molecule of claim 8 [or the vector of claim 17], or progeny of the host cell.

21. (Amended)

A plant, a plant part, a seed, a plant cell or progeny thereof comprising the recombinant nucleic acid molecule of claim 8 [or the vector of claim 17].

23. (Amended)

The plant, plant part, seed or plant cell of claim 21, wherein the plant, plant part, seed or plant cell is of a species selected from the group consisting of alfalfa, almond, apple, apricot, arabidopsis, artichoke, atriplex, avocado, barley, beet, birch, brassica, cabbage, cacao, cantalope, carnations, castorbean, caulifower, celery, clover, coffee, corn, cotton, cucumber, garlic, grape, grapefruit, hemp, hops, lettuce, maple, melon, mustard, oak, oat, olive, onion, orange, pea, peach, pear, pepper, pine, plum, poplar, potato, prune, radish, rape, rice, roses, rye, salicornia sorghum, soybean, spinach, squash, strawberries, sunflower, sweet corn, tobacco, tomato and wheat [of

potato, tomato, brassica, cotton, sunflower, strawberries, spinach, lettuce, rice, soybean, corn, wheat, rye, barley, atriplex, sorgum, alfalfa, salicornia and the plants in Table 5].

26. (Amended)

A method for producing a recombinant host cell [capable of expressing] that expresses [the] a nucleic acid molecule[of any of claims 1 to 4], the method comprising introducing into the host cell a vector of claim 17.

28. (Amended)

The method of claim [27]26, wherein the genome of the host cell also [includes] comprises a functional TNHX or PNHX gene.

29. (Amended)

The method of claim [27]26, wherein the genome of the host cell does not [include] comprise a functional TNHX or PNHX gene.

31. (Amended)

A method for expressing a TNHX or PNHX transporter polypeptide in the host cell of claim 19, [or the plant, plant part, seed or plant cell of claim 21,] the method comprising culturing the host cell under conditions suitable for gene expression.

53. (Amended)

A method of producing a genetically transformed plant which expresses or overexpresses a TNHX transporter polypeptide, a PNHX transporter polypeptide or a polypeptide having Na^+/H^+ transporter activity and [capable of increasing] provides increased salt tolerance in a cell and wherein the plant has increased salt tolerance, comprising:

(a) cloning or synthesizing a TNHX nucleic acid molecule, a PNHX nucleic acid molecule or a nucleic acid molecule which codes for a Na^+/H^+ transporter polypeptide, wherein the polypeptide is capable of providing salt tolerance to a plant;

(b) inserting the nucleic acid molecule in a vector so that the nucleic acid molecule is operably linked to a promoter;

(c) inserting the vector into a plant cell or plant seed;

(d) regenerating the plant from the plant cell or plant seed, wherein salt tolerance in the plant is increased compared to a wild type plant.

55. (Amended)

The nucleic acid molecule of claim 4, comprising [any one of] SEQ ID NO.1[, SEQ ID NO.3, SEQ ID NO.17, SEQ ID NO.19].